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(54) Title: ANTINEOPLASTIC COMBINATIONS SUCH AS RAPAMYCIN TOGETHER WITH GEMCITABINE OR FLUOROURACIL

(57) Abstract: The invention provides the use of a combination of an mTOR inhibitor such as a rapamycin and an antimetabolite antineoplastic agent such as gemcitabine or fluorouracil in the treatment of neoplasms.



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ANTINEOPLASTIC COMBINATIONS SUCH AS RAPAMYCIN TOGETHER WITH GEMCITABINE OR FLUOROURACIL

This invention relates to antineoplastic combinations, more particularly to the use of combinations of an mTOR inhibitor (e.g. rapamycin 42-ester with 3-hydroxy-2-
5 (hydroxymethyl)-2-methylpropionic acid (CCI-779)) and an antimetabolite antineoplastic agent in the treatment of neoplasms.

BACKGROUND OF THE INVENTION

10 Rapamycin is a macrocyclic triene antibiotic produced by Streptomyces hygroscopicus, which was found to have antifungal activity, particularly against Candida albicans, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S.N. Sehgal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31, 539 (1978); U.S. Patent 3,929,992; and U.S. Patent 3,993,749]. Additionally,
15 rapamycin alone (U.S. Patent 4,885,171) or in combination with picibanil (U.S. Patent 4,401,653) has been shown to have antitumor activity.

The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in
20 preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); R. Y. Calne et al., Lancet 1183 (1978); and U.S. Patent 5,100,899]. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the
25 formation of IgE-like antibodies.

Rapamycin is also useful in preventing or treating systemic lupus erythematosus [U.S. Patent 5,078,999], pulmonary inflammation [U.S. Patent 5,080,899], insulin dependent diabetes mellitus [U.S. Patent 5,321,009], skin disorders, such as psoriasis [U.S. Patent 5,286,730], bowel disorders [U.S. Patent
30 5,286,731], smooth muscle cell proliferation and intimal thickening following vascular injury [U.S. Patents 5,288,711 and 5,516,781], adult T-cell leukemia/lymphoma [European Patent Application 525,960 A1], ocular inflammation [U.S. Patent 5,387,589], malignant carcinomas [U.S. Patent 5,206,018], cardiac inflammatory disease [U.S. Patent 5,496,832], and anemia [U.S. Patent 5,561,138].

Rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid (CCI-779) is ester of rapamycin which has demonstrated significant inhibitory effects on tumor growth in both in vitro and in vivo models. The preparation and use of hydroxyesters of rapamycin, including CCI-779, are disclosed in U.S. Patent
5 5,362,718.

CCI-779 exhibits cytostatic, as opposed to cytotoxic properties, and may delay the time to progression of tumors or time to tumor recurrence. CCI-779 is considered to have a mechanism of action that is similar to that of sirolimus. CCI-779 binds to and forms a complex with the cytoplasmic protein FKBP, which inhibits an enzyme,
10 mTOR (mammalian target of rapamycin, also known as FKBP12-rapamycin associated protein [FRAP]). Inhibition of mTOR's kinase activity inhibits a variety of signal transduction pathways, including cytokine-stimulated cell proliferation, translation of mRNAs for several key proteins that regulate the G1 phase of the cell cycle, and IL-2-induced transcription, leading to inhibition of progression of the cell
15 cycle from G₁ to S. The mechanism of action of CCI-779 that results in the G₁ S phase block is novel for an anticancer drug.

In vitro, CCI-779 has been shown to inhibit the growth of a number of histologically diverse tumor cells. Central nervous system (CNS) cancer, leukemia (T-cell), breast cancer, prostate cancer, and melanoma lines were among the most
20 sensitive to CCI-779. The compound arrested cells in the G₁ phase of the cell cycle.

In vivo studies in nude mice have demonstrated that CCI-779 has activity against human tumor xenografts of diverse histological types. Gliomas were particularly sensitive to CCI-779 and the compound was active in an orthotopic glioma model in nude mice. Growth factor (platelet-derived)-induced stimulation of a human
25 glioblastoma cell line in vitro was markedly suppressed by CCI-779. The growth of several human pancreatic tumors in nude mice as well as one of two breast cancer lines studied in vivo also was inhibited by CCI-779.

DESCRIPTION OF THE INVENTION

30 This invention provides the use of combinations of an mTOR inhibitor and an antimetabolite antineoplastic agent as antineoplastic combination chemotherapy. In particular, these combinations are useful in the treatment of renal cancer, soft tissue cancer, breast cancer, neuroendocrine tumor of the lung, cervical cancer, uterine cancer, head and neck cancer, glioma, non-small lung cell cancer, prostate cancer,

pancreatic cancer, lymphoma, melanoma, small cell lung cancer, ovarian cancer, colon cancer, esophageal cancer, gastric cancer, leukemia, colorectal cancer, and unknown primary cancer. This invention also provides combinations of an mTOR inhibitor and an antimetabolite antineoplastic agent for use as antineoplastic combination chemotherapy, in which the dosage of either the mTOR inhibitor or the antimetabolite antineoplastic agent or both are used in subtherapeutically effective dosages.

As used in accordance with this invention, the term "treatment" means treating a mammal having a neoplastic disease by providing said mammal an effective amount of a combination of an mTOR inhibitor and an antimetabolite antineoplastic agent with the purpose of inhibiting growth of the neoplasm in such mammal, eradication of the neoplasm, or palliation of the mammal.

As used in accordance with this invention, the term "providing," with respect to providing the combination, means either directly administering the combination, or administering a prodrug, derivative, or analog of one or both of the components of the combination which will form an effective amount of the combination within the body.

mTOR is the mammalian target of rapamycin, also known as FKBP12-rapamycin associated protein [FRAP]. Inhibition of mTOR's kinase activity inhibits a variety of signal transduction pathways, including cytokine-stimulated cell proliferation, translation of mRNAs for several key proteins that regulate the G1 phase of the cell cycle, and IL-2-induced transcription, leading to inhibition of progression of the cell cycle from G1 to S.

mTOR regulates the activity of at least two proteins involved in the translation of specific cell cycle regulatory proteins (Burnett, P.E., PNAS 95: 1432 (1998) and Isotani, S., J. Biol. Chem. 274: 33493 (1999)). One of these proteins p70s6 kinase is phosphorylated by mTOR on serine 389 as well as threonine 412. This phosphorylation can be observed in growth factor treated cells by Western blotting of whole cell extracts of these cells with antibody specific for the phosphoserine 389 residue.

As used in accordance with this invention, an "mTOR inhibitor" means a compound or ligand which inhibits cell replication by blocking progression of the cell

cycle from G1 to S by inhibiting the phosphorylation of serine 389 of p70s6 kinase by mTOR.

The following standard pharmacological test procedure can be used to determine whether a compound is an mTOR inhibitor, as defined herein. Treatment of growth factor stimulated cells with an mTOR inhibitor like rapamycin completely blocks phosphorylation of serine 389 as evidenced by Western blot and as such constitutes a good assay for mTOR inhibition. Thus whole cell lysates from cells stimulated by a growth factor (eg. IGF1) in culture in the presence of an mTOR inhibitor should fail to show a band on an acrylamide gel capable of being labeled with an antibody specific for serine 389 of p70s6K.

Materials:

	NuPAGE LDS Sample Buffer	(Novex Cat # NP0007)
15	NuPAGE Sample Reducing Agent	(Novex Cat # NP0004)
	NuPAGE 4-12% Bis-Tris Gel	(Novex Cat # NP0321)
	NuPAGE MOPS SDS Running Buffer	(Novex Cat # NP0001)
	Nitrocellulose	(Novex Cat # LC2001)
	NuPAGE Transfer Buffer	(Novex Cat # NP0006)
20	Hyperfilm ECL	(Amersham Cat # RPN3114H)
	ECL Western Blotting Detection Reagent	(Amersham Cat # RPN2134)

Primary antibody: Phospho-p70 S6 Kinase (Thr389) (Cell Signaling Cat # 9205)
 Secondary antibody: Goat anti-rabbit IgG-HRP conjugate (Santa Cruz Cat # sc-2004)

25

Methods:

A. Preparation of Cell Lysates

Cell lines were grown in optimal basal medium supplemented with 10% fetal bovine serum and penicillin/treptomycin. For phosphorylation studies, cells were subcultured in 6-well plates. After the cells have completely attached, they were either serum-starved. Treatment with mTOR inhibitors ranged from 2 to 16 hours. After drug treatment, the cells were rinsed once with PBS (phosphate buffered saline without Mg⁺⁺ and Ca⁺⁺) and then lysed in 150-200 µl NuPAGE LDS sample buffer per well. The lysates were briefly sonicated and then centrifuged for 15 minutes at 14000 rpm. Lysates were stored at minus -80⁰C until use.

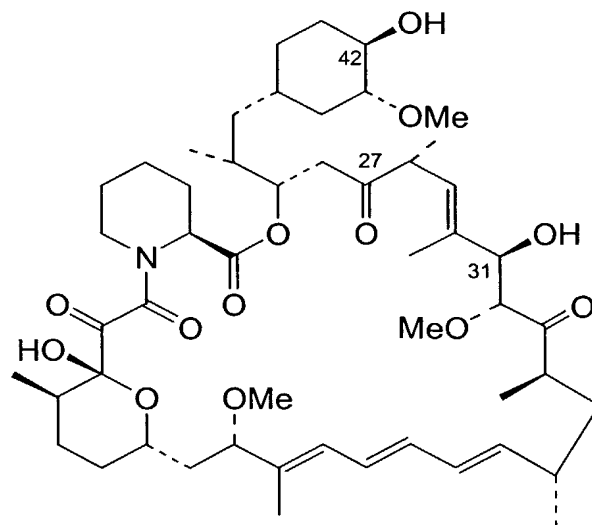
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The test procedure can also be run by incubating the cells in growth medium overnight, after they have completely attached. The results under both sets of conditions should be the same for an mTOR inhibitor.

5 B. Western Blot Analysis

- 1) Prepare total protein samples by placing 22.5 μ l of lysate per tube and then add 2.5 μ l NuPAGE sample reducing agent. Heat samples at 70⁰C for 10 minutes. Electrophoresed using NuPAGE gels and NuPAGE SDS buffers.
- 2) Transfer the gel to a nitrocellulose membrane with NuPAGE transfer buffer.
10 The membrane are blocked for 1 hour with blocking buffer (Tris buffered saline with 0.1%-Tween and 5% nonfat-milk). Rinse membranes 2x with washing buffer (Tris buffered saline with 0.1%-Tween).
- 3) Blots/membrane are incubated with the P-p70 S6K (T389) primary antibody (1:1000) in blocking buffer overnight at 4⁰ C in a rotating platform.
- 15 4) Blots are rinsed 3x for 10 minutes each with washing buffer, and incubated with secondary antibody (1:2000) in blocking buffer for 1 hour at room temperature.
- 5) After the secondary antibody binding, blots are washed 3x for 10 minutes each with washing buffer, and 2x for 1 minute each with Tris-buffered saline,
20 followed by chemiluminescent (ECL) detection and then exposed to chemiluminescence films.

As used in accordance with this invention, the term "a rapamycin" defines a class of immunosuppressive compounds which contain the basic rapamycin nucleus
25 (shown below). The rapamycins of this invention include compounds which may be chemically or biologically modified as derivatives of the rapamycin nucleus, while still retaining immunosuppressive properties. Accordingly, the term "a rapamycin" includes esters, ethers, oximes, hydrazones, and hydroxylamines of rapamycin, as well as rapamycins in which functional groups on the rapamycin nucleus have been
30 modified, for example through reduction or oxidation. The term "a rapamycin" also includes pharmaceutically acceptable salts of rapamycins, which are capable of forming such salts by virtue of containing either an acidic or basic moiety.



RAPAMYCIN

It is preferred that the esters and ethers of rapamycin are of the hydroxyl groups at the 42- and/or 31-positions of the rapamycin nucleus, esters and ethers of a hydroxyl group at the 27-position (following chemical reduction of the 27-ketone), and that the oximes, hydrazones, and hydroxylamines are of a ketone at the 42-position (following oxidation of the 42-hydroxyl group) and of 27-ketone of the rapamycin nucleus.

Preferred 42- and/or 31-esters and ethers of rapamycin are disclosed in the following patents, which are all hereby incorporated by reference: alkyl esters (U.S. Patent 4,316,885); aminoalkyl esters (U.S. Patent 4,650,803); fluorinated esters (U.S. Patent 5,100,883); amide esters (U.S. Patent 5,118,677); carbamate esters (U.S. Patent 5,118,678); silyl ethers (U.S. Patent 5,120,842); aminoesters (U.S. Patent 5,130,307); acetals (U.S. Patent 5,51,413); aminodiester (U.S. Patent 5,162,333); sulfonate and sulfate esters (U.S. Patent 5,177,203); esters (U.S. Patent 5,221,670); alkoxyesters (U.S. Patent 5,233,036); O-aryl, -alkyl, -alkenyl, and -alkynyl ethers (U.S. Patent 5,258,389); carbonate esters (U.S. Patent 5,260,300); arylcarbonyl and alkoxy carbonyl carbamates (U.S. Patent 5,262,423); carbamates (U.S. Patent 5,302,584); hydroxyesters (U.S. Patent 5,362,718); hindered esters (U.S. Patent 5,385,908); heterocyclic esters (U.S. Patent 5,385,909); gem-disubstituted esters (U.S. Patent 5,385,910); amino alkanolic esters (U.S. Patent 5,389,639); phosphorylcarbamate esters (U.S. Patent 5,391,730); carbamate esters (U.S. Patent

5,411,967); carbamate esters (U.S. Patent 5,434,260); amidino carbamate esters (U.S. Patent 5,463,048); carbamate esters (U.S. Patent 5,480,988); carbamate esters (U.S. Patent 5,480,989); carbamate esters (U.S. Patent 5,489,680); hindered N-oxide esters (U.S. Patent 5,491,231); biotin esters (U.S. Patent 5,504,091); O-alkyl ethers (U.S. Patent 5,665,772); and PEG esters of rapamycin (U.S. Patent 5,780,462). The preparation of these esters and ethers are disclosed in the patents listed above.

Preferred 27-esters and ethers of rapamycin are disclosed in U.S. Patent 5,256,790, which is hereby incorporated by reference. The preparation of these esters and ethers are disclosed in the patents listed above.

Preferred oximes, hydrazones, and hydroxylamines of rapamycin are disclosed in U.S. Patents 5,373,014, 5,378,836, 5,023,264, and 5,563,145, which are hereby incorporated by reference. The preparation of these oximes, hydrazones, and hydroxylamines are disclosed in the above listed patents. The preparation of 42-oxorapamycin is disclosed in 5,023,263, which is hereby incorporated by reference.

Particularly preferred rapamycins include rapamycin [U.S. Patent 3,929,992], CCI-779 [rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid; U.S. Patent 5,362,718], and 42-O-(2-hydroxy)ethyl rapamycin [U.S. Patent 5,665,772].

When applicable, pharmaceutically acceptable salts of the rapamycin can be formed from organic and inorganic acids, for example, acetic, propionic, lactic, citric, tartaric, succinic, fumaric, maleic, malonic, mandelic, malic, phthalic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, naphthalenesulfonic, benzenesulfonic, toluenesulfonic, camphorsulfonic, and similarly known acceptable acids when the rapamycin contains a suitable basic moiety. Salts may also be formed from organic and inorganic bases, such as alkali metal salts (for example, sodium, lithium, or potassium) alkaline earth metal salts, ammonium salts, alkylammonium salts containing 1-6 carbon atoms or dialkylammonium salts containing 1-6 carbon atoms in each alkyl group, and trialkylammonium salts containing 1-6 carbon atoms in each alkyl group, when the rapamycin contains a suitable acidic moiety.

It is preferred that the mTOR inhibitor used in the antineoplastic combinations of this invention is a rapamycin, and more preferred that the mTOR inhibitor is rapamycin, CCI-779, or 42-O-(2-hydroxy)ethyl rapamycin.

5 As described herein, CCI-779 was evaluated as a representative mTOR inhibitor in the mTOR inhibitor plus antimetabolite combinations of this invention.

 The preparation of CCI-779 is described in U.S. Patent 5,362,718, which is hereby incorporated by reference. When CCI-779 is used as an antineoplastic agent, it is projected that initial i.v. infusion dosages will be between about 0.1 and 100
10 mg/m² when administered on a daily dosage regimen (daily for 5 days, every 2-3 weeks), and between about 0.1 and 1000 mg/m² when administered on a once weekly dosage regimen. Oral or intravenous infusion are the preferred routes of administration, with intravenous being more preferred.

15 As used in accordance with this invention, the term "antimetabolite" means a substance which is structurally similar to a critical natural intermediate (metabolite) in a biochemical pathway leading to DNA or RNA synthesis which is used by the host in that pathway, but acts to inhibit the completion of that pathway (i.e., synthesis of DNA or RNA). More specifically, antimetabolites typically function by (1) competing with
20 metabolites for the catalytic or regulatory site of a key enzyme in DNA or RNA synthesis, or (2) substitute for a metabolite that is normally incorporated into DNA or RNA, and thereby producing a DNA or RNA that cannot support replication. Major categories of antimetabolites include (1) folic acid analogs, which are inhibitors of dihydrofolate reductase (DHFR); (2) purine analogs, which mimic the natural purines
25 (adenine or guanine) but are structurally different so they competitively or irreversibly inhibit nuclear processing of DNA or RNA; and (3) pyrimidine analogs, which mimic the natural pyrimidines (cytosine, thymidine, and uracil) but are structurally different so they competitively or irreversibly inhibit nuclear processing of DNA or RNA.

 The following are representative examples of antimetabolites of this invention.

30 5-Fluorouracil (5-FU; 5-fluoro-2,4(1*H*,3*H*)-pyrimidinedione) is commercially available in a topical cream (FLUOROPLEX or EFUDEX) a topical solution (FLUOROPLEX or EFUDEX), and as an injectable containing 50 mg/mL 5-fluorouracil (ADRUCIL or flurouracil).

Floxuradine (2'-deoxy-5-fluorouridine) is commercially available as an injectable containing 500 mg/vial of floxuradine (FUDR or floxuradine).

Thioguanine (2-amino-1,7-dihydro-6-*H*-purine-6-thione) is commercially available in 40 mg oral tablets (thioguanine).

- 5 Cytarabine (4-amino-1-(beta)-D-arabinofuranosyl-2(1H)-pyrimidinone) is commercially available as a liposomal injectable containing 10 mg/mL cytarabine (DEPOCYT) or as a liquid injectable containing between 1mg - 1g/vial or 20 mg/mL (cytarabine or CYTOSAR-U).

- 10 Fludarabine (9-*H*-Purin-6-amine,2-fluoro-9-(5-O-phosphono-(beta)-D-arabino-furanosyl) is commercially available as a liquid injectable containing 50 mg/vial (FLUDARA).

6-Mercaptopurine (1,7-dihydro-6*H*-purine-6-thione) is commercially available in 50 mg oral tablets (PURINETHOL).

- 15 Methotrexate (MTX; *N*-[4-[(2,4-diamino-6-pteridiny)l)methyl]methylamino]-benzoyl]-L-glutamic acid) is commercially available as a liquid injectable containing between 2.5 - 25 mg/mL and 20 mg - 1 g/vial (methotrexate sodium or FOLEX) and in 2.5 mg oral tablets (methotrexate sodium).

- 20 Gemcitabine (2'-deoxy-2',2'-difluorocytidine monohydrochloride ((beta)-isomer)), is commercially available as a liquid injectable containing between 200 mg - 1g/vial (GEMZAR).

Capecitabine (5'-deoxy-5-fluoro-*N*-[(pentyloxy)carbonyl]-cytidine) is commercially available as a 150 or 500 mg oral tablet (XELODA).

- 25 Pentostatin ((*R*)-3-(2-deoxy-(beta)-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol) is commercially available as a liquid injectable containing 10 mg/vial (NIPENT).

Trimetrexate (2,4-diamino-5-methyl-6-[(3,4,5-trimethoxyanilino)methyl]-quinazoline mono-D-glucuronate) is commercially available as a liquid injectable containing between 25 - 200 mg/vial (NEUTREXIN).

- 30 Cladribine (2-chloro-6-amino-9-(2-deoxy-(beta)-D-*erythro*pento-furanosyl)-purine) is commercially available as a liquid injectable containing 1 mg/mL (LEUSTATIN).

The following table briefly summarizes some of the recommended dosages for the antimetabolites listed above.

Drug	Dosage	Regimen
5-Fluorouracil	12 mg/kg oral 6 mg/kg oral 370 - 600 mg/m ² i.v.	daily for 4 days days 6, 8, 10, 12 no drug on days 5, 7, 9, and 11; doses cut in half if toxicity observed daily for 5 days, every 3-4 weeks
Floxuridine (FUDR)	0.1-0.6 mg/kg	daily by arterial infusion
Cytarabine (DEPOCYT)	50 mg	every 14 days for 5 doses during induction period; followed by every 28 days for maintenance
Cytarabine (injectable)	100 mg/m ² 2-3 g/m ²	daily for 7 days twice daily for 2-6 days
Fludarabine (FLUDARA)	25 mg/m ²	30 min infusion for 5 consecutive days; every 28 days
6-Mercaptopurine (PURINETHOL)	2.5-5 mg/kg 1.5-2.5 mg/kg	daily for induction daily for maintenance
Methotrexate	15-30 mg oral	daily for 5 day course; repeated 3-5 times
Gemcitabine (GEMZAR)	1000 mg/m ² /30 min 1000 -1250 mg/m ² /30 min	single agent: once weekly for 7 weeks, followed by 1 week rest, then once weekly for 3 out of every 4 weeks combination therapy: days 1, 8, 15 per 28 day cycle, or days 1 and 8 per 21 day cycle
Capecitabine (XELODA)	2500 mg/m ²	daily for 2 weeks followed by 1 week rest period
Pentostatin (NIPENT)	4 mg/m ²	as bolus injection or diluted as i.v. infusion; every other week
Trimetrexate (NEUTREXIN)	45 mg/m ²	i.v. infusion once daily for 21 days
Cladribine (LEUSTATIN)	0.09 mg/kg/day	continuous infusion for 7 consecutive days

This invention also covers the use of an mTOR inhibitor plus an antimetabolite in which a biochemical modifying agent is part of the chemotherapeutic regimen. The term "biochemical modifying agent" is well known and understood to those skilled in the art as an agent given as an adjunct to antimetabolite therapy, which serves to potentiate its antineoplastic activity, as well as counteract the side effects of the antimetabolite. Leucovorin and levofolinate are typically used as biochemical modifying agents for methotrexate and 5-FU therapy.

Leucovorin (5-formyl-5,6,7,8-tetrahydrofolic acid) is commercially available as an injectable liquid containing between 5 - 10 mg/mL or 50 - 350 mg/vial (leucovorin calcium or WELLCOVORIN) and as 5 - 25 mg oral tablets (leucovorin calcium).

Levofolinate (pharmacologically active isomer of 5-formyltetrahydrofolic acid) is commercially available as an injectable containing 25 - 75 mg levofolinate (ISOVORIN) or as 2.5 - 7.5 mg oral tablets (ISOVORIN).

Preferred mTOR inhibitor plus antimetabolite combinations of this invention include CCI-779 plus gemcitabine; CCI-779 plus 5-fluorouracil; and CCI-779 plus 5-fluorouracil plus leucovorin. It is preferred that the CCI-779 plus gemcitabine combination be used in treating pancreatic cancer and that the CCI-779 plus 5-fluorouracil combination (with or without leucovorin) be used in treating colorectal cancer.

The antineoplastic activity of the CCI-779 plus antimetabolite combination was confirmed in *in vitro* and *in vivo* standard pharmacological test procedures using combinations of CCI-779 plus gemcitabine; and CCI-779 plus 5-fluorouracil as representative combinations of this invention. The following briefly describes the procedures used and the results obtained.

Human rhabdomyosarcoma lines Rh30 and Rh1 and the human glioblastoma line SJ-GBM2 were used for *in vitro* combination studies with CCI-779 and antimetabolite agents. *In vivo* studies used a human neuroblastoma (NB1643) and human colon line GC3.

Dose response curves were determined for each of the drugs of interest. The cell lines Rh30, Rh1 and SJ-G2 were plated in six-well cluster plates at 6×10^3 , 5×10^3 and 2.5×10^4 cells/well respectively. After a 24 hour incubation period, drugs were added in either 10%FBS+RPMI 1640 for Rh30 and Rh1 or 15%FBS+DME for SJ-G2. After seven days exposure to drug containing media, the nuclei were released by treating the cells with a hypotonic solution followed by a detergent. The nuclei were then counted with a Coulter Counter. The results of the experiments were graphed and the IC_{50} (drug concentration producing 50% inhibition of growth) for each drug was determined by extrapolation. Because the IC_{50} s varied slightly from experiment to experiment, two values that bracketed the IC_{50} of each drug were used in the interaction studies. The point of maximum interaction between two drugs occurs

when they are present in a 1:1 ratio if the isobole is of standard shape. Therefore, each of the three approximate IC_{50} concentrations of CCI-779 was mixed in a 1:1 ratio with each of three approximated IC_{50} s of gemcitabine or 5-FU. This resulted in nine 1:1 combinations of drugs in each experiment plus three IC_{50} concentrations for CCI-779 and the other drug. This protocol usually resulted in at least one combination for each drug containing an IC_{50} value. The 1:1 combination of IC_{50} concentrations for CCI-779 and each chemotherapy drug was then used to calculate additivity, synergism, or antagonism using Berenbaum's formula: $x/X_{50} + y/Y_{50} = 1, <1, >1$. If the three concentrations of CCI-779 tested alone didn't produce an IC that matched any of the three ICs of the other compound tested alone, all the 1:1 combinations were checked to see if their ICs fell between the appropriate ICs of drugs tested singly. If they did, the effect was considered additive.

The results obtained in the *in vitro* standard pharmacological test procedure showed that in no case did the combinations yield less than a 50% inhibition of growth indicating that the combinations were at least additive and produced no evidence of antagonism.

Female CBA/CaJ mice (Jackson Laboratories, Bar Harbor, ME), 4 weeks of age, were immune-deprived by thymectomy, followed 3 weeks later by whole-body irradiation (1200 cGy) using a ^{137}Cs source. Mice received 3×10^6 nucleated bone marrow cells within 6-8 h of irradiation. Tumor pieces of approximately 3 mm^3 were implanted in the space of the dorsal lateral flanks of the mice to initiate tumor growth. Tumor-bearing mice were randomized into groups of seven prior to initiating therapy. Mice bearing tumors each received drug when tumors were approximately 0.20-1 cm in diameter. Tumor size was determined at 7-day intervals using digital Vernier calipers interfaced with a computer. Tumor volumes were calculated assuming tumors to be spherical using the formula $[(\pi/6) \times d^3]$, where d is the mean diameter. CCI-779 was given on a schedule of 5 consecutive days for 2 weeks with this cycle repeated every 21 days for 3 cycles. This resulted in CCI-779 being given on days 1-5, 8-12 (cycle 1); 21-25, 28-32 (cycle 2); and 42-46, 49-53 (cycle 3). The schedule of the other chemotherapy drug for each study was as follows:

Gemcitabine on days 1, 4, 8 in cycle 1 only

The combination of CCI-779 and gemcitabine was evaluated in a human colon (GC3) mouse xenograft test procedure. In this test procedure, CCI-779 was given daily x 5 for 2 consecutive weeks every 21 days for 3 cycles and gemcitabine given on days 1, 4, and 8 in the first cycle only. The presence of CCI-779 did not enhance tumor regression seen in the first cycle with gemcitabine treatment. However, groups treated with CCI-779 were delayed in the time required to reach 2-3x the original pretreatment tumor volume (versus gemcitabine alone), indicating that there was at least an additive benefit derived from the combination treatment.

Based on the results of these standard pharmacological test procedures, combinations of an mTOR inhibitor plus an antimetabolite chemotherapeutic agent are useful as antineoplastic therapy. More particularly, these combinations useful in treating treatment of renal carcinoma, soft tissue sarcoma, breast cancer, neuroendocrine tumor of the lung, cervical cancer, uterine cancer, head and neck cancer, glioma, non-small cell lung cancer, prostate cancer, pancreatic cancer, lymphoma, melanoma, small cell lung cancer, ovarian cancer, colon cancer, esophageal cancer, gastric cancer, leukemia, colorectal cancer, and unknown primary cancer. As these combinations contain at least two active antineoplastic agents, the use of such combinations also provides for the use of combinations of each of the agents in which one or both of the agents is used at subtherapeutically effective dosages, thereby lessening toxicity associated with the individual chemotherapeutic agent.

In providing chemotherapy, multiple agents having different modalities of action are typically used as part of a chemotherapy "cocktail." It is anticipated that the combinations of this invention will be used as part of a chemotherapy cocktail that may contain one or more additional antineoplastic agents depending on the nature of the neoplasia to be treated. For example, this invention also covers the use of the mTOR inhibitor/antimetabolite combination used in conjunction with other chemotherapeutic agents, such as alkylating agents (i.e., cisplatin, carboplatin, streptazoin, melphalan, chlorambucil, carmustine, methchloroethamine, lomustine, bisulfan, thiotepa, ifofamide, or cyclophosphamide); hormonal agents (i.e., estramustine, tamoxifen, toremifene, anastrozole, or letrozole); antibiotics (i.e., plicamycin, bleomycin, mitoxantrone, idarubicin, dactinomycin, mitomycin, doxorubicin, or daunorubicin); immunomodulators (i.e., interferons, IL-2, or BCG);

antimitotic agents (i.e., vinblastine, vincristine, teniposide, or vinorelbine); topoisomerase inhibitors (i.e., topotecan, irinotecan, or etoposide); and other agents (i.e., hydroxyurea, trastuzumab, altretamine, retuximab, paclitaxel, docetaxel, L-asparaginase, or gemtuzumab ozogamicin).

5

As used in this invention, the combination regimen can be given simultaneously or can be given in a staggered regimen, with the mTOR inhibitor being given at a different time during the course of chemotherapy than the antimetabolite. This time differential may range from several minutes, hours, days, weeks, or longer
10 between administration of the two agents. Therefore, the term combination does not necessarily mean administered at the same time or as a unitary dose, but that each of the components are administered during a desired treatment period. The agents may also be administered by different routes. For example, in the combination of an mTOR inhibitor plus an antimetabolite, it is anticipated that the mTOR inhibitor will be
15 administered orally or parenterally, with parenterally being preferred, while the antimetabolite may be administered parenterally, orally, or by other acceptable means. For the CCI-779 combination with gemcitabine, it is preferred that the gemcitabine be administered parenterally. For the CCI-779 combination with 5-FU and leucovorin, it is preferred that the 5-FU and leucovorin are administered
20 parenterally. These combination can be administered daily, weekly, or even once monthly. As typical for chemotherapeutic regimens, a course of chemotherapy may be repeated several weeks later, and may follow the same timeframe for administration of the two agents, or may be modified based on patient response.

Accordingly this invention also provides a product comprising an mTOR
25 inhibitor and an antimetabolite antineoplastic agent as a combined preparation for simultaneous, separate or sequential use in the treatment of a neoplasm in a mammal.

As typical with chemotherapy, dosage regimens are closely monitored by the treating physician, based on numerous factors including the severity of the disease,
30 response to the disease, any treatment related toxicities, age, health of the patient, and other concomitant disorders or treatments.

Based on the results obtained with the representative CCI-779 plus antimetabolite combinations, it is projected that the initial i.v. infusion dosage of the mTOR inhibitor will be between about 0.1 and 100 mg/m², with between about 2.5

and 70 mg/m² being preferred. It is also preferred that the mTOR inhibitor be administered by i.v., typically over a 30 minute period, and administered about once per week. The initial dosages of the antimetabolite component will depend on the component used, and will be based initially on physician experience with the agents chosen.

Based on the results obtained with the CCI-779 plus antimetabolite combinations, it is projected that for the mTOR inhibitor plus gemcitabine combination, the initial i.v. infusion dosage of the mTOR inhibitor will be between about 0.1 and 100 mg/m², with between about 2.5 and 70 mg/m² being preferred, and the initial i.v. infusion dosage of gemcitabine will be between about 400 and 1500 mg/m², with between about 800 and 1000 mg/m² being preferred. It is initially projected that patients will receive a 30 minute i.v. infusion of the mTOR inhibitor, followed immediately or preceded by a 30 minute i.v. infusion of gemcitabine on days 1 and 8 of a 21 day treatment cycle. After one or more treatment cycles, the dosages can be adjusted upwards or downwards depending on the results obtained and the side effects observed.

Based on the results obtained, when CCI-779 is used in combination with 5-FU and leucovorin, it is projected that in an mTOR inhibitor plus 5-FU plus leucovorin regimen, the initial i.v. infusion dosage of the mTOR inhibitor will be between about 0.1 and 100 mg/m², with between about 2.5 and 70 mg/m² being preferred; the initial i.v. infusion dosage of leucovorin will be between about 50 and 500 mg/m², with about 200 mg/m² being preferred; and the initial i.v. infusion dosage of 5-FU will be between about 500 and 7500 mg/m², with between about 1000 and 5000 mg/m² being preferred. It is initially projected that the combination will be administered according to the following regimen: patients will receive a 1 hour i.v. infusion of leucovorin once weekly during each 6 week treatment cycle; immediately following each dose of leucovorin, 5-FU is administered as a 24-hour continuous i.v. infusion. The mTOR inhibitor will be administered beginning on day 8, of cycle 1, and will be given once weekly as a 30 minute i.v. infusion. Each 6 week treatment cycle is followed by a 1 week rest before beginning the next 6 week treatment cycle. After one or more treatment cycles, the dosages can be adjusted upwards or downwards depending on the results obtained and the side effects observed.

For commercially available antimetabolites, the existing dosage form can be used, with the dosages divided as need be. Alternatively, such agents or antimetabolites that are not commercially available can be formulated according to standard pharmaceutical practice. Oral formulations containing the active compounds of this invention may comprise any conventionally used oral forms, including tablets, capsules, buccal forms, troches, lozenges and oral liquids, suspensions or solutions. Capsules may contain mixtures of the active compound(s) with inert fillers and/or diluents such as the pharmaceutically acceptable starches (e.g. corn, potato or tapioca starch), sugars, artificial sweetening agents, powdered celluloses, such as crystalline and microcrystalline celluloses, flours, gelatins, gums, etc. Useful tablet formulations may be made by conventional compression, wet granulation or dry granulation methods and utilize pharmaceutically acceptable diluents, binding agents, lubricants, disintegrants, surface modifying agents (including surfactants), suspending or stabilizing agents, including, but not limited to, magnesium stearate, stearic acid, talc, sodium lauryl sulfate, microcrystalline cellulose, carboxymethylcellulose calcium, polyvinylpyrrolidone, gelatin, alginic acid, acacia gum, xanthan gum, sodium citrate, complex silicates, calcium carbonate, glycine, dextrin, sucrose, sorbitol, dicalcium phosphate, calcium sulfate, lactose, kaolin, mannitol, sodium chloride, talc, dry starches and powdered sugar. Preferred surface modifying agents include nonionic and anionic surface modifying agents. Representative examples of surface modifying agents include, but are not limited to, poloxamer 188, benzalkonium chloride, calcium stearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, magnesium aluminum silicate, and triethanolamine. Oral formulations herein may utilize standard delay or time release formulations to alter the absorption of the active compound(s). The oral formulation may also consist of administering the active ingredient in water or a fruit juice, containing appropriate solubilizers or emulsifiers as needed.

In some cases it may be desirable to administer the compounds directly to the airways in the form of an aerosol.

The compounds may also be administered parenterally or intraperitoneally. Solutions or suspensions of these active compounds as a free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary

conditions of storage and use, these preparation contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

For the purposes of this disclosure, transdermal administrations are understood to include all administrations across the surface of the body and the inner linings of bodily passages including epithelial and mucosal tissues. Such administrations may be carried out using the present compounds, or pharmaceutically acceptable salts thereof, in lotions, creams, foams, patches, suspensions, solutions, and suppositories (rectal and vaginal).

Transdermal administration may be accomplished through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semi-permeable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

Suppository formulations may be made from traditional materials, including cocoa butter, with or without the addition of waxes to alter the suppository's melting point, and glycerin. Water soluble suppository bases, such as polyethylene glycols of various molecular weights, may also be used.

CLAIMS

What is claimed is:

1. A method of treating a neoplasm in a mammal in need thereof, which
5 comprises providing to said mammal an effective amount of a combination comprising
an mTOR inhibitor and an antimetabolite antineoplastic agent.
2. A method of treating a neoplasm according to claim 1 wherein either the
mTOR inhibitor, the antimetabolite, or both are provided in subtherapeutically effective
10 amounts.
3. A method according to claim 2 in which the mTOR inhibitor is provided
in a subtherapeutically effective amount.
- 15 4. A method according to claim 2 or claim 3 in which the antimetabolite is
provided in a subtherapeutically effective amount.
5. A method according to any one of claims 1 to 4, wherein the neoplasm
is one of the following:
20 renal cancer; soft tissue sarcoma; breast cancer; a neuroendocrine tumor of the lung;
cervical cancer; uterine cancer;. a head and neck cancer; glioma; non-small cell lung
cancer; prostate cancer; pancreatic cancer; lymphoma; melanoma; small cell lung
cancer; ovarian cancer; colon cancer; esophageal cancer; gastric cancer; leukemia;
colorectal cancer; or unknown primary cancer.
25
6. A method according to any one of claims 1 to 5, wherein the
combination further comprises a biochemical modifying agent.
7. A method according to claim 6, wherein the biochemical modifying
30 agent is leucovorin or levofolinate.
8. A method according to any one of claims 1 to 5 in which the
antimetabolite is gemcitabine.

9. A method according to claim 8, wherein the neoplasm is pancreatic cancer.

10. A method according to any one of claims 1 to 5 in which the
5 antimetabolite is 5-fluorouracil.

11. A method according to claim 10, in which the combination further comprises a biochemical modifying agent.

10 12. A method according to any one of claims 1 to 5 which comprises providing to said mammal an effective amount of a combination of an mTOR inhibitor, 5-fluorouracil, and leucovorin.

15 13. A method according to any one of claims 10 to 12, wherein the neoplasm is colorectal cancer.

14. A method according to any one of claims 1 to 13, wherein the mTOR inhibitor is a rapamycin.

20 15. A method according to claim 14, wherein the rapamycin is rapamycin.

16. A method according to claim 14, wherein the rapamycin is 42-O-(2-hydroxy)ethyl rapamycin.

25 17. A method according to claim 14, wherein the rapamycin is rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid.

18. An antineoplastic combination which comprises an effective amount of an mTOR inhibitor and an antimetabolite antineoplastic agent.

30 19. The combination of claim 18, which further comprises a biochemical modifying agent.

20. The combination according to claim 19, wherein the mTOR inhibitor is a rapamycin.

21. The combination according to claim 19, wherein the rapamycin is rapamycin.

22. The combination according to claim 19, wherein the rapamycin is 42-O-(2-hydroxy)ethyl rapamycin.

23. The combination according to claim 19, wherein the rapamycin is rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid.

24. A product comprising an mTOR inhibitor and an antimetabolite antineoplastic agent as a combined preparation for simultaneous, separate or sequential use in the treatment of a neoplasm in a mammal.

25. A product as claimed in claim 24 further comprising a biochemical modifying agent.

26. A product as claimed in claim 24 or claim 25 wherein the neoplasm is one of the following:
renal cancer; soft tissue sarcoma; breast cancer; a neuroendocrine tumor of the lung; cervical cancer; uterine cancer; a head and neck cancer; glioma; non-small cell lung cancer; prostate cancer; pancreatic cancer; lymphoma; melanoma; small cell lung cancer; ovarian cancer; colon cancer; esophageal cancer; gastric cancer; leukemia; colorectal cancer; or unknown primary cancer.

27. A product as claimed in any one of claims 24 to 26. wherein the mTOR inhibitor is a rapamycin.

28. A product according to claim 27, wherein the rapamycin is rapamycin.

29. A product according to claim 27, wherein the rapamycin is 42-O-(2-hydroxy)ethyl rapamycin.

30. A product according to claim 27, wherein the rapamycin is rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid.

5 31. A product according to any one of claims 24 to 30 in which the antimetabolite is gemcitabine or 5-fluorouracil.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 02/10912

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K45/06 A61K31/436 A61K31/7068 A61K31/513 A61K31/519
 A61P35/00 //(A61K31/7068,31:436),(A61K31/7068,31:519,31:436),
 (A61K31/513,31:436),(A61K31/519,31:513,31:436)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, MEDLINE, BIOSIS, CHEM ABS Data, WPI Data, PAJ, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 52514 A (ELI LILLY GES MBH ;KONWALINKA GUENTHER (AT); MARGREITER RAIMUND (A) 21 October 1999 (1999-10-21) claims 6-15	1-9, 14, 18-21, 24-28, 31 1-31
Y	----	
X	US 5 066 493 A (SEHGAL SURENDRA N ET AL) 19 November 1991 (1991-11-19)	1-6, 10-15, 18-21, 24-28, 31 1-31
Y	claims ----- -/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

* & * document member of the same patent family

Date of the actual completion of the international search

8 August 2002

Date of mailing of the international search report

26/08/2002

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 02/10912

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ENG C P ET AL: "Activity of rapamycin (AY-22,989) against transplanted tumors." THE JOURNAL OF ANTIBIOTICS. JAPAN OCT 1984, vol. 37, no. 10, October 1984 (1984-10), pages 1231-1237, XP001098253 ISSN: 0021-8820 abstract	1-6, 10-15, 18-21, 24-28,31
Y	abstract	1-31
Y	----- ALEXANDRE J ET AL: "LA RAPAMYCINE ET LE CCI-779//RAPAMYCIN AND CCI-779" CANCER BULLETIN, MEDICAL ARTS PUB, HOUSTON, US, vol. 10, no. 86, October 1999 (1999-10), pages 808-811, XP001078856 ISSN: 0008-5448 abstract	1-31
Y	----- ALEXANDRE J ET AL: "PHASE I STUDY OF CCI-779, A NOVEL RAPAMYCIN ANALOG: PRELIMINARY RESULTS" PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, NEW YORK, NY, US, vol. 41, no. 41, March 2000 (2000-03), page 613 XP001071217 ISSN: 0197-016X abstract	1-31
P,X	----- PUNT C J A ET AL: "A phase I study of escalating doses of CCI-779 in combination with 5-fluorouracil and leucovorin in patients with advanced solid tumors." EUROPEAN JOURNAL OF CANCER, vol. 37, no. Supplement 6, October 2001 (2001-10), page S17 XP001093978 11th European Cancer Conference;Lisbon, Portugal; October 21-25, 2001, October, 2001 ISSN: 0959-8049 abstract	1-7, 10-31
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 02/10912

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>BONI J ET AL: "Pharmacokinetics of escalating doses of CCI-779 in combination with 5-fluorouracil and leucovorin in patients with advanced solid tumors." EUROPEAN JOURNAL OF CANCER, vol. 37, no. Supplement 6, October 2001 (2001-10), page S68 XP001093977 11th European Cancer Conference;Lisbon, Portugal; October 21-25, 2001, October, 2001 ISSN: 0959-8049 abstract</p> <p>-----</p>	1-7, 10-31

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box I.1

Although claims 1-17 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

Continuation of Box I.2

The subject-matter of present claims is defined by means of the functional features: "mTOR inhibitor" and "antimetabolite antineoplastic agent"

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

international application No.
PCT/US 02/10912

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: —
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.: —
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 02/10912

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9952514	A	21-10-1999	WO 9952514 A2 21-10-1999
			AU 3126799 A 01-11-1999
			BR 9909617 A 12-12-2000
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			TR 200002969 T2 22-01-2001
			TR 200101633 T2 21-06-2002
			TW 466112 B 01-12-2001
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			US 4885171 A 05-12-1989
			BE 877700 A1 14-01-1980
			JP 55073616 A 03-06-1980
			ZA 7905449 A 26-11-1980